investigated via the construction of a Markov chain including all states assumed by the coupled kinesin-microtubule system, and mechanical rate constants optimized to fit experimental data. Further analysis with kinetic Monte Carlo yields sidestepping probabilities for each kinesin species. We also report preliminary results on the use of motion planning methods for simulating the geometry and dynamics of the protein/tubulin/obstacle system, using coarse-graining of PDB protein structures. The results of these simulations are compared to the Markov chain results, and used to reconcile and refine both methods.

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The Kinesin-8 Kif18B uses a Non-Canonical Form of Directed Motility to Target the Extreme Microtubule Plus-End

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A defining feature of kinesins is that they are motile, using ATP-hydrolyzed energy to translocate along the microtubule (MT) lattice. The motile properties of kinesins can define their subcellular distributions. For example, the mitotic kinesin-8 Kif18A uses high processivity to enriches at the plus-end of long stable MTs. In contrast, the localization of Kif18B, a second kinesin-8, is governed by its binding to EB1, a MT plus-end tracking protein. It is therefore unclear whether Kif18B requires plus-end directed motility to accumulate at MT plus-ends. Using structured illumination microscopy (SIM), we show that a sub-population of Kif18B occupies the extreme tip of MT plus-ends ahead of EB1. This observation raises the possibility that Kif18B uses plus-end directed motility to ''sample'' protofilaments corresponding to the GTP cap. Using single molecule assays, we show that Kif18B is not highly processive, and that the motor switches frequently between plus-end directed and diffusive modes of motility. Diffusion is promoted by the tail of Kif18B. The tail of Kif18B is therefore multi-functional: it allows the motor to interact with EB1, and we show here that it also contains a second MT binding site, a property that increases the MT on-rate of the motor. Our mean squared displacement analysis shows that the speed of ATP-driven plus-end motility of Kif18B is well below the velocity of growing MT plus-ends. However, computer simulations suggest that a combination of directed motility and diffusion allows Kif18B to outpace a growing MT plus-end. Collectively, our work demonstrates that the motile properties of Kif18B deviate significantly from conventional transport motors. Instead, Kif18B is designed to efficiently explore the GTP cap, enabling it to promote catastrophes and thereby regulate MT length.

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Motility of Kinetochore Kinesin CENP-E is Enhanced by Tubulin Detyrosination

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Targeted transport by intracellular motors can be regulated by posttranslational modifications of polymerized tubulins in the microtubule tracks, but little is known about such effects for motors that drive chromosome motions during mitosis. Microtubules that form mitotic spindle are differentially modified at the C-terminal residue of α-tubulin: polymers that point to the spindle equator, but not the astral microtubules, are preferentially detyrosinated. Here we examine the influence of tubulin detyrosination on CENP-E, the kinetochore-localized kinesin-7 that transports pole-proximal chromosomes to the spindle equator. We polymerized purified human tubulins that were fully tyrosinated or detyrosinated, and examined the suitability of these tracks for motility of recombinant GFP-tagged CENP-E motor. Using fluorescence microscopy we show that single molecules of CENP-E walk faster and more processively on detyrosinated microtubules. Moreover, on these tracks the CENP-E motor can generate larger force than on the tyrosinated microtubules, as determined using stationary optical trap. On both types of microtubules CENP-E took 8-nm steps, exhibited similar dwell times and frequencies of backward stepping. However, motor's detachment increased with resisting force faster when CENP-E was walking on tyrosinated microtubules, leading to the detachment from these polymers at on average smaller load, 4.5 pN vs. 6.4 pN for detyrosinated microtubules. The enhanced motility of CENP-E motor on detyrosinated microtubules, most notably its ability to carry a larger load, could potentially explain the targeted transport of mitotic chromosomes toward the spindle equator.

Cell Mechanics, Mechcanosensing, and Motility I

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Concentration Profiles of Actin-Binding Molecules in Lamellipodia with Retrograde Flow

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Motile cells form flat protrusions in the direction of motion called lamellipodia. The actin filament network inside lamellipodia flows opposite to the direction of motion (retrograde flow) due to actin polymerization at the front. Hence, actin binding molecules are subject to transport to the rear in the bound state and diffuse freely in the unbound state. We analyse this non-linear reaction-diffusion-advection process with respect to the concentration profiles of these species and provide analytic approximations for the profiles. Retrograde flow may cause a depletion zone of actin-binding molecules close to the leading edge. The existence of such zone depends on the free molecule concentration in the cell body, on the ratio of the diffusion length to the distance bound molecules travel rearward with the retrograde flow before dissociating, and the ratio of the diffusion length to the width of the region with retrograde flow and actin binding. Our calculations suggest the existence of depletion zones for the F-actin cross-linkers filamin and α-actinin in 3T3-fibroblasts, which is in line with the small elastic moduli of the F-actin network close to the leading edge found in measurements of the force motile cells are able to exert.

693-Pos Board B473

Helical Buckling in Filopodia

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Filopodia are thin actin rich membrane protrusion that allow cells to interactively probe their environment by periodic protrusion and shrinkage interrupted by occasional kinks. Filopodial actin is thought to play a pivotal role in filopodial force transduction, bending, and rotation. We investigated whether, and how, actin within filopodia is responsible for filopodia dynamics by conducting simultaneous force spectroscopy and confocal fluorescent imaging of F-actin in membrane protrusions. The actin shafts frequently undergo buckling and rotational motion which was correlated with retrograde movement of actin inside the filopodium. Pulling on an object attached to the filopodium tip strongly correlated with the presence of actin near the tip region and pulling forces were found to be correlated with movement of buckles along the actin shaft. We propose a mechanism that is based on accumulation of torsional twist in the rotating actin shaft and consequently leads to torsional induced buckling and shortening of the actin shaft.

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Characteristics of Cell Shape in Two Dimensions

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The shape of a cell is closely related with its properties, and in certain conditions it has been shown that change in cell shape can change cell behavior and outcome. However in two dimensions cells have complicated outlines, and it is not yet clear how best to characterize cell shape. Here we study the characteristics of cell shape of the 10T1/2 cell line in two-dimensional culture, and a few osteosarcoma cancer cell lines. The two dimensional surfaces are treated to make them either hydrophobic or hydrophilic, and the cells imaged after fixing, using membrane and actin labeling. The cells are also treated with pharmacological modulators of the cytoskeleton, yielding a large number of different shape types, and shape perturbations. Shape parameters are calculated by first using image processing to obtain binary outlines of the cells, and then calculating a number of geometric parameters, such as area, perimeter

length, aspect ratio, fractal dimension etc. Simultaneously we also use orthogonal polynomial decompositions using Zernike moments to calculate shape characteristics. Using statistical data analysis we compare the ability of the Zernike moment expansion to capture the different shapes and their perturbations with that of the geometric parameters. We find that both types of shape calculations give insights into how a cell determines its shape on a surface.

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Does Cell Shape Determine Cell Fate?

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Different types of cells, i.e. from different tissues, typically look quite different from each other. Even when cultured on two-dimensional surfaces like glass slides or tissue culture polystyrene under identical conditions, cells adopt different shapes. These shapes are in general functions of the cytoskeletal properties of those cells, itself a subset of what we call the ''state'' of the cell. Moreover the changes in cell shape upon perturbation of the surface or of the cells themselves should reflect their intrinsic cellular properties, i.e. the cell state. Significant evidence has accumulated that changes in shape can also alter cellular properties, at least for some cells. Our experiments suggest that for Mesenchymal Stem Cells (MSCs), shape perturbations have consequences for their differentiation into osteoblasts. Thus shape seemed linked to fate. These statements beg the question: is it possible to use cell shape to assess cell state? For example can we back-calculate the cytoskeletal properties of the cell from the way it looks on surfaces? This question becomes all the more interesting for cancer cells since cancer cells are known to have altered mechanical properties compared to normal cells, and invasive cancer cells appear to have altered mechanical properties compared to non-invasive cancer cells. In this work we present a combination of experiments and statistical data analysis to try to begin to understand how cell shapes are affected by changes in surface properties or by perturbations of the cytoskeleton. We use fluorescent imaging to obtain the two-dimensional profile of cells and novel Third Harmonic Generation methods to obtain three-dimensional images on cells on substrates. We use these experiments to infer how the cell shape of cancer cells could be associated with their invasive properties. We discuss some rudimentary mathematical models based on these results.

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Real-Time Deformability Cytometry: High-Throughput Mechanical Phenotyping for Changes in Cell Function

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Changes in cell function are often accompanied by alterations of the intrinsic cell structure particularly the cytoskeleton. This leads to distinct mechanical changes. For example, cells become softer during malignant transformation and stiffer during differentiation. Exploiting the mechanical phenotype of cells as an inherent, label-free marker requires a high-throughput and robust measurement technique. Here, we introduce real-time deformability cytometry (RT-DC) for mechanical single cell classification of heterogeneous cell populations at rates of several hundred cells per second in real-time. Performing RT-DC on primary human hematopoietic stem cells and mature blood cells we demonstrate its capability to detect lineage and source specific mechanical phenotypes. We also find that different stages of the cell cycle possess a unique mechanical fingerprint allowing the distinction between cells in G2 and M phase. In summary, RT-DC represents a novel flow cytometric approach that enables the translation of mechanical phenotyping from basic research into applications in biology and medicine.

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Cortical Actin Tension, Elastic Modulus and Cytosolic Pressure in Fibroblasts Determined using Atomic Force Microscopy

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The cortical actin cytoskeleton lies just beneath the cell membrane to define cell shape and mechanical properties and thus plays a key role in cell biology processes such as migration and morphogenesis. The organization of actin filaments and actomyosin contractility are known to contribute to modifying the mechanical properties of the cortex. However, recent work report how these properties contribute to cortex tension and intracellular pressure. Here we propose a new method for using an atomic force microscope to determine actin cortex mechanical properties of non-adherent human foreskin fibroblasts including the cortex tension and intracellular pressure, but additionally, the cortex elastic modulus which has not been measured before. First, we validated the method by measuring the surface tension of water-in-oil microdrops deposited on a glass surface. We extracted an average tension of T~20.2 nN/μm, which agrees with macroscopic experimental methods. We then proceeded to measure cortical actin mechanical properties in non-adherent fibroblasts, and compare this to the properties after inducing two perturbations (i) adding blebbistatin which inhibits myosin II molecular motor activity, and (ii) adding CK-666 which inhibits Arp2/3-mediated actin branching. Our results show that perturbing the actin cortex had significant changes in each of the cortical mechanical properties: blebbistatin reduced them by ~50%, while CK-666 increased them by ~2-fold. These results validate our novel method for determining the quantitative mechanics of the actin cortex in eukaryotic cells.

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Relating Local Nanomechanical Response of Cells to Intracellular Forces and Cell Morphology

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Mechanical processes regulate cell physiology primarily at the molecular level, but current techniques have difficulty achieving mechanical contrast at molecular length scales. We recently developed an AFM-based tool that can probe mechanical properties of living cells with nanoscale resolution. However, the measurements we obtained at the nanoscale are hard to reconcile with the viscoelastic view of cell mechanical behavior. We predominantly observe elastic response with little hysteresis in the corresponding force distance curves. In addition, force distance curves are surprisingly linear, which would not be the case for viscoelastic materials indented by conical AFM tips. We have created a model for the nanomechanical response of cells that takes intracellular forces into account. The model not only explains the near-elastic response and the linearity of force distance curves, but also makes quantitative predictions about cell shape and its relationship to the local nanomechanical response. We experimentally tested and verified these predictions on cells exhibiting different morphologies. In addition to these predictions, the model allows determining intracellular forces from the AFM images, such as tension across actin fibers and cortex tension. This work expands the existing cell mechanical models into the nanoscale and enables AFM to obtain physiologically relevant parameters from mechanical images.

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Co-Culture Changes the Mechanical Properties of Cells

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Using an Atomic Force Microscope (AFM) with a 5.3 um diameter spherical probe we are measuring the elastic modulus of human mammary epithelial cells (HMEC) as they are co-cultured with immortal, tumorigenic, and finally metastatic. We are performing measurement over both normal cells and cancer cells. In order study the change induced by the co-culturing, our measurements will include the cell-pairs (normal cells and neighboring cancer cells).
So far, we found that normal cells show a significant difference in modulus after co-culturing with cancer cells. Measurements to date indicate that the moduli of HMEC increased more than 20% after co-culture with metastatic cells for 6 hours. We expect to report moduli under similar conditions for immortalized and tumorigenic HMEC cells and metastatic cells known as MDA-MB-231. In addition, we observe modulus differences due to transfection treatments. We also plan to report on these differences for immortalized, tumorigenic and metastatic versions of HMEC cells.
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Quantitation of Compositional Changes in the Non-Erythroid Membrane Skeleton due to External Forces

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The spectrin based membrane skeleton plays important roles in the mechanical and biological functions of living cells. Unlike the membrane skeleton of red blood cells, its non-erythroid counterpart has seen very little attention. Yet, it